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Large-scale meridional and zonal variability in the nitrogen isotopic composition of plankton in the Atlantic Ocean

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ABSTRACT

The zonal (*ca.* 15º-40ºW along 26-29ºN) and meridional (*ca.* 30ºN-30ºS along 28º-29ºW) variability of $\delta^{15}N$ of suspended particles and zooplankton (>40 µm) was studied to assess the influence of nitrogen fixation in the isotopic budget of the tropical and subtropical Atlantic ocean. Two cruises were conducted in October-November 2007 and April-May 2008 comprising a zonal and meridional transect each. In the region between 30º-15ºN, the concurrently measured nitrogen fixation was insufficient to explain the consistent patch of suspended particles with $\delta^{15}N < 2‰$ and points to a significant contribution of atmospheric deposition of light nitrogen to the isotopic budget. The equatorial region (15ºN-10ºS) is subject to intense nitrogen fixation, which, according to a two-end-member mixing model, may explain 40-60% of the observed $\delta^{15}N$ in suspended particles and 3-30% in zooplankton. In the South region between 10ºS-30ºS, low values (<4‰) were measured in suspended particles and zooplankton during 2008. The values of $\delta^{15}N$ of suspended particles suggest that nitrogen fixation, which is usually low (<10 µmol N m^{-2} d^{-1}), may represent 50-60% of phytoplankton nitrogen in this region. Hence, diazotrophy in the South Atlantic may be more important than previously thought.

INTRODUCTION

In many marine ecosystems, primary production is limited by the availability of nitrogen (Vitousek and Howarth, 1991; Karl et al., 1992; Moore et al., 2013). Reactive nitrogen is supplied to the euphotic zone by different physical, chemical and biological processes such as advective diffusion, atmospheric deposition and biological nitrogen fixation. The latter is mediated by organisms and, in the oligotrophic regions of the oceans, is a relevant source of new nitrogen (Paerl and Zehr, 2000). The ratio of stable isotopes in phytoplankton (^{15}N:^{14}N expressed as $\delta^{15}N$ in ‰) is variable, due to the contrasting preferences of the organisms for each isotope. The metabolic pathways usually discriminate against the heavy isotope (^{15}N), a discrimination that is measured by the isotopic fractionation factor (Montoya, 2008). Besides, the different forms of inorganic nitrogen have distinct signatures of $\delta^{15}N$. Deep-nitrate typically ranges between 3-6‰ (Montoya, 2008), atmospheric dinitrogen is, by definition, 0‰, and deep-ammonium lies between 6-8‰ (Miyake and Wada, 1967). Hence, a very different $\delta^{15}N$ of organic matter is expected, according to the source of nitrogen, if this is completely consumed. The isotopic signature of phytoplankton will depend then on the signature of the source of nitrogen and the degree of fractionation during uptake. Yet, the interpretation of $\delta^{15}N$ is not so straightforward. In the case of animals (*i.e.* upper trophic levels), a trophic effect is also observed whereby the tissues of the consumer are usually 2-4‰ heavier than the food, whereas the animal's excreta, mainly in the form of ammonium, can be 2-4‰ lighter than the food (Montoya, 2008; and references herein). In addition, cultured cyanobacteria growing on excess nitrate showed a strong fractionation factor, yielding $\delta^{15}N$ values similar to those produced by growth on dinitrogen (Bauersachs et al., 2009).

In the Atlantic Ocean, experimental data retrieved during large-scale surveys show that *Trichodesmium*, the most well-studied diazotroph, is distributed preferentially between 0-20ºN (Tyrrell et al., 2003; Moore et al, 2009; Fernández et al., 2010; Luo et al., 2012). In addition, nitrogen fixation, mostly measured with the method of Montoya et al. (1996), is more significant between 0º-15ºN (Moore et al., 2009; Fernández et al., 2010; Luo et al., 2012). The $\delta^{15}N$ of diazotrophs usually ranges between −1‰ and −2‰ (Montoya et al., 2002). However, the measured isotopic signature of nitrogen in suspended particles and the biogeochemical estimates of excess nitrogen available in the literature suggest that nitrogen fixation is more relevant in a region further north, between 15º-30ºN (Gruber and Sarmiento, 1997; Mahaffey et al., 2003; Mahaffey et al., 2004; Reynolds et al., 2007; Hansell et al., 2004). The time scales reflected by these measurements are different: *in situ* nitrogen fixation rates generally represent instantaneous rates over a few hours to 1 day, while $\delta^{15}N$ and excess nitrogen are indicators of the diazotrophic activity over longer periods of days to months. However, the determinants of this disagreement remain undefined. Duce

et al. (2008) argued that the atmospheric deposition of reactive nitrogen in the oceans has increased due to human activities and is fast approaching the marine N_2 fixation budget. Other studies have also shown an increase of the atmospheric deposition of ^{15}N-depleted nitrogen in high and temperate latitudes (Hastings et al., 2009; Mara et al., 2009; Morin et al., 2009; Holtgrieve et al., 2011), as a result of the increasing anthropogenic production of reactive nitrogen and/or natural speciation processes. In addition, Baker et al. (2007) and Knapp et al. (2010) reported depositional fluxes of low $\delta^{15}N$ similar to measured N_2 fixation rates in the Atlantic Ocean.

As part of a wider project, we have previously described the latitudinal and longitudinal distribution of measured community nitrogen fixation in the tropical and subtropical Atlantic Ocean (Fernández et al., 2010; Fernández et al., 2013) and the relative contribution of nitrogen fixation and nitrate eddy diffusion in supplying new nitrogen to the euphotic layer (Mouriño-Carballido et al., 2011). Here we report on the distribution of $\delta^{15}N$ in suspended particles and two size-fractions of plankton, with the aim of describing the large-scale latitudinal variability of nitrogen isotopic signatures in the Atlantic Ocean and comparing these inferred patterns of diazotrophy with concurrent, direct measurements of *in situ* N_2 fixation rates.

METHODS

Sampling, hydrography and chlorophyll a

Two research cruises were conducted in the tropical and subtropical Atlantic Ocean during 17 November-8 December 2007 and 13 April-2 May 2008 on board the BIO 'Hespérides'. The tracks followed by the vessel comprised a zonal and a meridional transect in each season (Fig. 1).

The vertical distribution of temperature, salinity, dissolved oxygen and fluorescence was measured by a SBE 911plus CTD attached to a rosette equipped with 12-L Niskin bottles which was fired to 300m depth, always before dawn. The vertical profiles of fluorescence and oxygen at each station were used to choose the sampling depths for the determination of inorganic nutrients concentration, chlorophyll *a* concentration, community $^{15}N_2$ fixation and natural abundance of nitrogen isotopes in suspended particles.

The concentration of chlorophyll *a* was measured at 6-7 depths distributed through the euphotic layer. At each depth a 250-mL sample was filtered, using low vacuum pressure, through 0.2 μm pore-size polycarbonate filters. The pigments were extracted overnight in 90% acetone at -4ºC. Fluorescence was subsequently measured on board with a Turner Designs 700 fluorometer, calibrated with pure chlorophyll *a* (Fluka).

Rates of N_2 fixation by the whole planktonic community in a 24-hour incubation period were determined in each station at the surface (5m), an intermediate depth (30-80m) and the depth of the deep chlorophyll maximum (DCM), and are already described in Fernández et al. (2010) and Fernández et al. (2013). Briefly, we incubated triplicate samples following the Montoya et al. (1996) protocol for the $^{15}N_2$-uptake technique with the modifications of Rees et al. (2009). The equations of Weiss (1970) and Montoya et al. (1996) were used to calculate the initial N_2 concentration (assuming equilibrium with atmosphere) and N_2 fixation rates, respectively. The limit of detection, estimated following Montoya et al. (1996), was 0.001 µmol N m^{-3} d^{-1}.

Natural abundance of nitrogen isotopes in suspended particles

For the determination of $\delta^{15}N$ signature in suspended particles ($\delta^{15}N_{sp}$), 2-L samples were taken at 6 depths through the euphotic layer in each pre-dawn station and filtered through a 25-mm diameter GF/F filter (Whatman). All filters were dried at 40ºC during 24 h and then stored until pelletization in tin capsules. The measurement of particulate organic nitrogen (PON) and ^{15}N atom% was carried out with an elemental analyzer combined with a continuous-flow stable isotope mass-spectrometer (FlashEA112 + Deltaplus, ThermoFinnigan) and using an acetanilide standard as reference. The limit of detection of the equipment was 0.20 µg N.

The isotopic signature observed in the suspended particles may be affected by the presence of other types of material in addition to phytoplankton (*i.e.* bacteria, detritus, zooplankton). The existence of a relationship between the particulate organic nitrogen (PON) to chlorophyll *a* (chl-*a*) ratio and the $\delta^{15}N$ of suspended particles is an indicator of such a trophic effect (Waser et al., 2000). The Pearson product-moment correlation coefficient of PON:chl-*a* and $\delta^{15}N_{sp}$ was calculated to test this possibility.

The weighted mean of $\delta^{15}N$ of suspended particles in the euphotic layer was used as an integral of the signature of phytoplankton in the euphotic zone to simplify the comparison with the $\delta^{15}N$ of the two size-fractions of zooplankton (40-200 µm and >200 µm). It was calculated, following Landrum et al. (2011), as:

$$\text{Weighted mean } \delta^{15}N_{sp} = \frac{\sum_i \left([PN]_i \times \Delta z_i \times \delta^{15}N_{sp} \right)}{\sum_i \left([PN]_i \times \Delta z_i \right)}$$

Where $[PN]_i$ is the concentration (µM) of particulate nitrogen, $\delta^{15}N_{sp}$ is the nitrogen isotopic composition of suspended particles (^{15}N:^{14}N, ‰), and Δz_i is the depth interval (m).

The fraction contribution of diazotroph nitrogen to the bulk suspended particles defined by Montoya et al. (2002) was also calculated as:

$$\%\ diazotroph\ N = 100 \times \left(\frac{\delta^{15}\mathrm{N_{sp}} - \delta^{15}\mathrm{NO_3^-}}{\delta^{15}\mathrm{N_{diazotroph}} - \delta^{15}\mathrm{NO_3^-}} \right)$$

Where $\delta^{15}N_{diazotroph}$ is the nitrogen isotopic composition of diazotrophs (^{15}N:^{14}N, ‰) and $\delta^{15}NO_3^-$is the nitrogen isotopic composition of deep-nitrate (^{15}N:^{14}N, ‰). As pointed by these authors, this two-end-member mixing model is sensitive to the values of the end members chosen ($\delta^{15}N_{diazotroph}$ and $\delta^{15}NO_3^-$). In order to represent only the nitrate in the upper thermocline, and avoid the effect of recently fixed nitrogen recycled between the upper water column and the thermocline in the calculations, the $\delta^{15}NO_3^-$ used was 4.5‰, which is the global average of deep-nitrate (Liu and Kaplan, 1989; Sigman et al., 1997). Due to the fact that most of our stations are oligotrophic, no additional fractionation factor during nitrate uptake was added. As a conservative choice representing the least contribution of nitrogen fixers, and considering the fact that little fractionation occurs during N_2 fixation (Montoya, 2007), the $\delta^{15}N_{diazotroph}$ used was −2‰ (Montoya et al., 2002).

Natural abundance of nitrogen isotopes in plankton

At each pre-dawn station, zooplankton were collected by vertical tows of a 40µm net of 30cm in diameter through the upper 200m of the water column at a constant towing speed of 60 m min^{-1}. The content of the collector was suspended in 500 mL of 20 µm-filtered seawater. Two 60 mL sub-samples were preserved, one in Lugol's solution and the other in formaldehyde, for the determination of abundance of *Trichodesmium* and other plankton by microscopical examination. *Trichodesmium* trichomes were more abundant in the fraction 40-200µm while colonies were present in the >200µm fraction. The rest of the sample was separated into two size fractions by passage through nylon sieves of 40 and 200µm. Each fraction was then re-suspended in 200 mL of 20µm-filtered seawater and subsequently filtered in pre-weighted 45-mm diameter GF/F filters by low vacuum pressure. All filters were dried for 24 h at 40ºC and stored until measurement of particulate organic nitrogen and ^{15}N atom% as previously described.

The relative contribution of diazotroph N to zooplankton biomass was estimated following Montoya et al. (2002) two-end-member mixing model for zooplankton:

$$\%\ diazotroph\ N = 100 \times \left(\frac{\delta^{15}\mathrm{N_{plankton}} - \delta^{15}\mathrm{N_{reference\ pl}}}{\delta^{15}\mathrm{N_{diazotroph}} - \delta^{15}\mathrm{N_{reference\ pl}}} \right)$$

Where $\delta^{15}N_{plankton}$ stands for the nitrogen isotopic composition of the plankton size-fraction (^{15}N:^{14}N, ‰), and $\delta^{15}N_{reference\ pl}$ is the $\delta^{15}N$ of reference zooplankton. Again, a conservative value of −2‰ was used for $\delta^{15}N_{diazotroph}$. The $\delta^{15}N$ of the reference plankton was calculated as the mean of the $\delta^{15}N_{40}$ or $\delta^{15}N_{200}$ measured in the stations where the lowest abundance of *Trichodesmium* and

nitrogen fixation were found, i.e., the stations between 0-20ºS in the latitudinal leg of 2007 cruise, where no influence of nitrogen fixation in the samples is expected. The values used were: 4.6‰ for the $\delta^{15}N_{reference\ 40}$, and 5.9‰ for $\delta^{15}N_{reference\ 200}$. This model is based on the use of reference plankton to account for the trophic effect, *i.e.*, the reference plankton serves as a proxy in both terms of the calculation; therefore, no additional fractionation term for the trophic effect was needed. The assumptions that are implied are: i) the size distribution of grazers in the sample and the reference plankton are similar, ii) the trophic fractionation in the sample and the reference is similar, and iii) in both locations the isotopic composition of the nitrate supporting the food web is the same (J. P. Montoya, Atlanta, personal communication).

RESULTS

Hydrography and fluorescence

In the latitudinal transects, the Equatorial upwelling was clearly defined by the rising of the isolines of temperature (T) and salinity (S) in both cruises (Fig. 2a, b, c, d). In order to simplify the analysis of data, we use the changes in the depth of the 16ºC isotherm, above and below 150m, to identify the area affected by the Equatorial upwelling and delimit three main regions along the transects, *i.e.*, North gyre (29º-15ºN), equatorial region (15ºN-10ºS) and South gyre (10º-33ºS).

The hydrographic settings found in these regions were similar in both legs. Surface waters in the equatorial region were always warmer (> 24ºC) and less saline (< 35 psu) than in the gyres in both seasons. In turn, the stability of the water column in the gyres was weaker than that found in the equatorial region, where the average Brunt-Väisäla frequency in the upper 125m was higher (Fernández et al., 2010). The fluorescence profiles showed a well-defined deep chlorophyll maximum (DCM) associated with the thermocline in both transects (Fig. 2e, f). This DCM was shallower and better defined in the equatorial region than in the gyres. By contrast, in the longitudinal sections, waters were warmer and slightly more saline in autumn 2007 than in spring 2008 (Fig. 2e), leading to a stronger stability of the water column, as indicated by the higher Brunt-Väisäla frequency measured in this cruise (Fernández et al., 2013). The DCM was located at *ca.* 100m in both zonal legs and no apparent trend in depth was observed (Fig. 2e, f).

Stable nitrogen isotopes in suspended particles ($\delta^{15}N_{sp}$) and particulate organic nitrogen (PON)

The Pearson product-moment correlation coefficient of PON:chl-*a* and $\delta^{15}N_{sp}$ showed no significant relationship, neither positive nor negative, in our two cruises (Fig. 3). The PON:chl-*a* ratio represents the contribution of other components of the food web than phytoplankton. If any

relationship between PON:chl-*a* ratio and $\delta^{15}N_{sp}$ is found, this will suggest a significant effect of detrital matter and/or other non-phytoplanktonic organisms on the observed signal of suspended particles. Thus, we can assume that the $\delta^{15}N$ of suspended particles in our data mainly reflects the composition of phytoplankton (Waser et al., 2000).

The zonal distribution of PON showed no apparent trend and the measured concentrations were similar in magnitude in both cruises (Fig. 4b, d), without any significant differences between cruises (t-test, n=60). The mean PON concentrations for the zonal transects were 0.22±0.12 µM in 2007 and 0.24±0.06 µM in 2008. In the latitudinal sections, the measured concentrations in the South gyre were similar in both cruises, showing similar values and vertical variability (Fig. 4a, c). In the North gyre and equatorial regions, measured PON differed significantly between cruises (t-test, $p < 0.01$, n=54 and $p < 0.05$, n=84, respectively). In the North gyre region, the concentrations of PON in 2007 cruise were higher than that of 2008 cruise. In the equatorial region, PON depicted a decreasing pattern in 2007 cruise, but no linear trend was observed in 2008.

In the zonal transect of 2007 cruise, the $\delta^{15}N_{sp}$ increased sharply by 4-6‰ from 80-100m to the base of the euphotic layer, probably reflecting the influence of the African upwelling in the easternmost stations (Fig. 5a). However, the $\delta^{15}N_{sp}$ distributed uniformly in the euphotic layer in 2008 cruise (Fig. 5b). In the latitudinal transects, the $\delta^{15}N_{sp}$ was lower in 2007 than in 2008, both in the North gyre (Fig 5c, d) and equatorial region (Fig 5e, f). By contrast, in the South gyre region, the vertical distribution of $\delta^{15}N_{sp}$ differed between stations in each cruises (Fig 5g, h), with a wide range of values between −4 and 4‰. The difference between cruises were significant in the zonal transect, the North gyre and the equatorial region (t-test, $p < 0.01$, n=72), but not in the South gyre region.

The correlations of $\delta^{15}N$ of suspended particles with ammonium concentration and with nitrate concentration are shown in Table 1. Considering all the stations in each cruise, $\delta^{15}N_{sp}$ correlated with ammonium concentration in 2007 ($p < 0.05$, n=128) and with nitrate in 2008 ($p < 0.05$, n=119).

Nitrogen isotopic signature in the euphotic layer

In order to compare the $\delta^{15}N$ of suspended particles and plankton net tows, we calculated the weighted mean of $\delta^{15}N$ of suspended particles to obtain an integrative $\delta^{15}N_{sp}$ signature for the whole euphotic layer. The patterns described by the $\delta^{15}N$ of the planktonic 40-200 µm ($\delta^{15}N_{40}$) and >200 µm ($\delta^{15}N_{200}$) size-fractions were very similar in all stations, with a few exceptions in the equatorial region and the zonal legs, and closely matched that of $\delta^{15}N_{sp}$ (Fig. 6). The average differences between $\delta^{15}N_{sp}$ and the two plankton size fractions were in the range previously described

(Minagawa and Wada, 1984): between the $\delta^{15}N_{sp}$ and the $\delta^{15}N_{40}$ that difference was 3.2‰ in 2007 and 2.6‰ in 2008; between the $\delta^{15}N_{sp}$ and the $\delta^{15}N_{200}$ was 4.3‰ in 2007 and 1.9‰ in 2008.

In the autumn 2007 meridional transect, the isotopic signature of suspended particles showed two minima (< −2‰) in the North gyre and South gyre regions. In the equatorial region, $\delta^{15}N_{sp}$ oscillated around 0‰ (Fig. 6). The $\delta^{15}N_{40}$ and $\delta^{15}N_{200}$ roughly followed these patterns. By contrast, the distributions were dome-shaped in spring 2008, reaching peak values in the equatorial region. In both cruises, the gyres presented low $\delta^{15}N$ values in most of the stations. Besides, a positive statistical correlation between $\delta^{15}N$ in the three fractions suggests a regular impact of light nitrogen across trophic levels (Table 2).

A two-way factorial ANOVA indicated significant differences between regions and cruises, and for $\delta^{15}N_{40}$ and $\delta^{15}N_{200}$, a significant interaction region-cruise, which enhances the difference (Table 3). The differences between regions appeared to be significant only for the North gyre-equatorial region (post-hoc Tukey HSD test), as can be also seen in figure 6.

We tried to estimate if *Trichodesmium* could be the major influence on the patterns observed but no significant correlation (Pearson's r) was found between the measured filament abundance (Fernández et al., 2010; Fernández et al., 2013) and the $\delta^{15}N$ of suspended particles (p=n.s., n=42), the 40-200 µm (p=n.s., n=41) or the >200 µm plankton size-fractions (p=n.s., n=42).

Nitrogen fixation rates (Fig. 6) were previously reported in Fernández et al. (2010) and Fernández et al. (2013). Briefly, in the longitudinal transects no apparent trend was depicted in 2007, while a clear increasing pattern to the East appeared in 2008 (Fig. 6b, d). In the spring 2008 zonal leg, the average vertically integrated N_2 fixation was 7-fold higher than that of autumn 2007 (8.3±3.3 µmol N m^{-2} d^{-1} *vs*. 1.2±0.5 µmol N m^{-2} d^{-1}). In both meridional transects, the highest integrated rates (*ca*. 250 and 150 µmol N m^{-2} d^{-1} in 2007 and 2008, respectively) were measured at stations located within the equatorial region (Fig. 6a, c). Besides, the North gyre showed higher diazotrophic activities than the South gyre. But, while N_2 fixation south of the Equator was almost undetectable during the 2007 cruise, substantial rates were measured in the Southern Hemisphere in 2008 (Fig. 6a, c).

Diazotroph nitrogen contribution to $\delta^{15}N$ in the euphotic layer

The contribution of diazotrophs to the observed $\delta^{15}N$ of suspended particles, 40-200µm and >200 µm plankton size-fractions, estimated by the two-end-member mixing models, decreased to the South in 2007 cruise (Table 4). In 2008, the minimum was observed in the equatorial region (Table 4). The importance of this contribution is higher in 2007, except in the South gyre, where the

contribution of diazotroph nitrogen was higher in all size-fractions. In both cruises, diazotrophy explains, on average, 61% of the observed $\delta^{15}N_{sp}$; 27% of $\delta^{15}N_{40}$, and 30% of $\delta^{15}N_{200}$.

DISCUSSION

Our data contributes to the existing studies in the Atlantic Ocean (Waser et al., 2000; Mino et al., 2002; Montoya et al., 2002; Mahaffey et al., 2003; Mahaffey et al., 2004; Reynolds et al., 2007; Landrum et al., 2011; Mompeán et al., 2013) providing basin-scale distribution of $\delta^{15}N$ in suspended particles and two plankton size-fractions during two contrasting seasons. We found a consistent ^{15}N-depleted signal (<4‰) in suspended particles ($\delta^{15}N_{sp}$) in the euphotic layer in most of the stations (Fig. 5; Fig. 6). This implies that nitrogen fixation and/or atmospheric deposition were supplying an important fraction of new nitrogen in most of the tropical and subtropical Atlantic Ocean in our cruises. The trends in the $\delta^{15}N$ of the two plankton size-fractions closely matched that of suspended particles, indicating a sensible impact of light nitrogen even in upper trophic levels, at leat for some regions (Fig. 6, Table 2).

In the meridional transect of 2008, the $\delta^{15}N$ signal in the >200µm size-fraction ($\delta^{15}N_{200}$) was lower than that in the 40-200 µm size-fraction ($\delta^{15}N_{40}$) in most of the stations (Fig. 6), contrary to the usual observed enrichment in ^{15}N of upper trophic levels (Montoya, 2008). One possible reason is the longer turnover times of mesozooplankton relative to phytoplankton and microplankton that could result in the uncoupling of different size-fractions, producing this inversion of the expected increasing pattern (Landrum et al., 2011; Mompeán et al., 2013). Other possible explanation could be the presence of *Trichodesmium* colonies, whicht were large enough to be retained in this size fraction, and would have lowered the isotopic signature of zooplankton.

Zonal and meridional variations in $\delta^{15}N$ in the North gyre

In the North gyre, the difference in $\delta^{15}N_{sp}$ between stations was higher in autumn 2007 than in spring 2008 (6‰ and 2‰, respectively). However, the vertical change in $\delta^{15}N_{sp}$ at each station was small in both cruises (Fig. 5), and the measured values of $\delta^{15}N_{sp}$ were always <2‰. Our data are in agreement with previous reports in the subtropical North Atlantic which show a range of variation between −2 and 4‰ in the signature of suspended particles (Montoya et al., 2002; Mahaffey et al., 2003; Reynolds et al., 2007; Landrum et al., 2011). These authors described a consistently depleted signal between 7º and 32ºN, which is also confirmed by geochemical tracers (Gruber and Sarmiento, 1997; Hansell et al., 2004) that point to a persistent excess nitrate relative to phosphate in this area, indicative of intense nitrogen fixation. These light nitrogen signatures have been

associated with a large impact of diazotrophic nitrogen in the isotopic budget of this area, discarding the influence of other sources of new light nitrogen because of their weak strength or unlikely occurrence (Reynolds et al., 2007; Landrum et al., 2011). However, later studies provided new insights that suggest a more relevant influence of alternative sources such as the atmospheric deposition of ^{15}N-depleted nitrogen (Baker et al., 2007; Hastings et al., 2009; Morin et al., 2009; Mara et al., 2009; Knapp et al., 2010; Holtgrieve et al., 2011; Mouriño-Carballido et al., 2011)

The δ^{15}N of deep-water nitrate typically ranges between 3-6‰ with a global average of 4.8‰ (Montoya, 2008). In the presence of excess nitrate, the isotopic fractionation, due to the incomplete exhaustion of the nitrate pool by phytoplankton ,could result in values of $\delta^{15}N_{sp}$ lower than 3‰ (Montoya, 2008). A recent study showed that cyanobacteria, especially *Trichodesmium*, growing on nitrate could express a nitrogen isotopic signal similar to that of nitrogen fixation depending on the isotopic composition of the nitrogen source, the degree of fractionation, and the species of cyanobacterium (Bauersachs et al., 2009). However, no excess dissolved inorganic nitrogen was found in surface waters in our zonal or meridional legs, where the concentration of nitrate in the euphotic layer was always lower than 130 nM (Mouriño-Carballido et al., 2011; Fernández et al., 2010; Fernández et al., 2013). We also recorded measurable but low abundances of *Trichodesmium* (<60 trichomes L^{-1}) in the euphotic layer (Fernández et al., 2010; Fernández et al., 2013), which is also an indication of potential diazotrophy in the area. But, no significant correlation appeared between *Trichodesmium* abundances and the $\delta^{15}N_{sp}$ (Pearson's r). Hence, we would not expect that a strong isotopic fractionation associated with cyanobacteria or other phytoplankters was responsible for the observed $\delta^{15}N_{sp}$ during our cruises.

The lack of data on atmospheric deposition of nitrogen during our study limits any direct comparison with the measured nitrogen fixation and the distribution of the δ^{15}N signature, but we can attempt to use an indirect analysis instead. The two-end-member model proposed by Montoya et al. (2002) yields a contribution of N_2 fixation to $\delta^{15}N_{sp}$ in the range 81-85% in 2007 and 59-61% in 2008 (Table 4), which is close to the previous estimation of 74% by Reynolds et al. (2007). However, experimental measurements of community nitrogen fixation in this region indicate modest rates of diazotrophy (<60 µmol N m^{-2} d^{-1}) throughout the year (Fig. 6; Moore et al., 2009; Benavides et al., 2011). During the spring 2008 cruise, Mouriño-Carballido et al. (2011) calculated the relative importance of nitrate eddy diffusion and measured rates of nitrogen fixation as sources of new nitrogen to the euphotic layer in the North gyre. They estimated that the average contribution of nitrogen fixation in this cruise was only 2% over daily time-scales. We acknowledge that the comparison of these two fluxes is difficult as they represent different time-scales, *i.e.*, the $\delta^{15}N_{sp}$ represents time-scales of days to weeks, while the measured nitrogen fixation time-scale is

one day. But the difference between the fluxes was 30-fold in 2008. This suggests that other sources than diazotrophy may be contributing to our observed $\delta^{15}N_{sp}$, and that the low values are not only a consequence of intense nitrogen fixation. The importance of the atmospheric deposition of low $\delta^{15}N$ nitrogen, natural or anthropogenic, is increasing in high and temperate latitudes (Hastings et al., 2009; Morin et al., 2009; Mara et al., 2009; Holtgrieve et al., 2011). According to the model of Duce et al. (2008), the atmospheric supply of anthropogenic reactive nitrogen in the central North Atlantic is usually higher in the latitudinal range between 5º and 25ºN. The $\delta^{15}N$ of this anthropogenic N depends on its origin and is extremely variable (Fang et al., 2011). For instance, the $\delta^{15}N$ of fuel NO_x produced by power plants ranges between 5 and 13‰ (Heaton, 1990; Kiga et al., 2000), but that of thermal NO_x produced by vehicle exhausts ranges between −13 and −2‰ (Heaton, 1990). Besides, in the Atlantic Ocean between 45ºN-45ºS, Morin et al. (2009) found a $\delta^{15}N$ of atmospheric nitrate that ranged between −7 and −1.6‰ and was mainly representing natural sources. In the Mediterranean Sea (Crete Island), Mara et al. (2009) described a consistent source of low $\delta^{15}N$ nitrate throughout the year with a potential impact on the isotopic budget of intermediate and deep waters, which could lead to an overestimation of N_2 fixation if atmospheric nitrate is neglected. In spite of that, previous studies discarded the effect of this process in the analysis of the nitrogen isotopic budget, based on the assumption that the flux is small compared to nitrogen fixation (Landrum et al., 2011) or to the export flux of nitrogen out of the euphotic layer (Reynolds et al., 2007). By contrast, Baker et al. (2007) and Knapp et al. (2010) measured atmospheric depositional fluxes of ^{15}N-depleted nitrogen similar to those of N_2 fixation in the North Atlantic Ocean. Considering this information, we suggest that the observed $\delta^{15}N_{sp}$ in the North gyre region during our cruises could be the result of the supply of light nitrogen through both nitrogen fixation and atmospheric deposition. Therefore, discarding the effect of this atmospheric supply in the analysis of $\delta^{15}N_{sp}$ would result in the overestimation of nitrogen fixation.

The signature of light nitrogen found in suspended spread over the food web. Firstly, the $\delta^{15}N$ distributions of the two zooplankton size-fractions and the suspended particles are significantly correlated (Fig. 6, Table 2). Secondly, the observed difference between plankton and particles at each station (2-4‰) is in agreement with previous studies reporting that zooplankton nitrogen is typically 3‰ heavier than phytoplankton (Minagawa and Wada, 1984) due to the enrichment of zooplankton tissues by the isotopic fractionation associated with metabolic and excretory processes (Montoya, 2008). Thirdly, both plankton size-fractions showed relatively low values of $\delta^{15}N$ throughout the transects (<4‰). Again, the data provided by the two-end-member model proposed by Montoya et al. (2002) point out that the contribution of nitrogen fixation to upper trophic levels in 2007 represents 48-52% in the 40-200µm size-fraction and 41-43% in the >200 µm size-fraction (Table 4). In 2008, it represented roughly 16-21% of the 40-200 µm fraction signal and the 31-36%

of the >200 µm size-fraction (Table 4). However, we would expect an overestimation of this contribution due to the combined effect of atmospheric deposition of ^{15}N-depleted nitrogen and nitrogen fixation in the isotopic budget of this region.

The $\delta^{15}N$ measured in suspended particles and zooplankton suggests a consistent supply of light nitrogen in this region of the Atlantic Ocean throughout the year, which coincides with previous studies (Montoya et al., 2002; Mahaffey et al., 2003; Reynolds et al., 2007; Landrum et al., 2011). However, we did not measure the isotopic composition of the depositional fluxes, which could be compared with measured community nitrogen fixation in our cruises to determine the actual contribution of each flux (Baker et al., 2007; Knapp et al., 2010). Further studies, characterizing the strength, frequency and $\delta^{15}N$ of the atmospheric sources of nitrogen relative to *in situ* measured nitrogen fixation, will help to unequivocally ascertain the relative importance of each process in determining the $\delta^{15}N$ signatures in the North Atlantic.

Meridional variations in $\delta^{15}N$ in the equatorial region

In the equatorial region (15ºN-10ºS), the meridional trends of the $\delta^{15}N$ of suspended particles coincided with those previously described by Mahaffey et al. (2004). However, our absolute values are lower than theirs, in the range −2 to 4‰, and closer to those measured by Reynolds et al. (2007) in the water column and by Mino et al. (2002) in surface waters. The upwelling in this region allows a persistent diffusion of deep nitrate to surface waters, which was reflected in the increase of nitrate concentration during our cruises (Fernández et al., 2010; Mouriño-Carballido et al., 2011; Fernández et al., 2013), and is likely to support a substantial fraction of primary production. Therefore, heavy deep-nitrate is probably determining part of the $\delta^{15}N$ of suspended particles in the equatorial region.

In autumn 2007, the difference between the $\delta^{15}N$ of 40-200 µm and >200 µm plankton size-fractions (2‰) suggests either a different time scale in the integration of the signal or a low efficiency in the transference of nitrogen to upper trophic levels. The latter could be attributed to the loss of isotopically light ammonium through excretory processes, which was suggested as a major source of light nitrogen in oligotrophic regions (Checkley and Miller, 1989; Montoya, 2008). However, the positive correlation between $\delta^{15}N$ of suspended particles and ammonium concentration in our cruises (Table 1) suggests that the increase in ammonium is increasing the $\delta^{15}N_{sp}$ and may not be related to the excretion of plankton.

The cyanobacterium *Trichodesmium* exudates up to 50% of recent fixed N_2 as dissolved organic nitrogen, which can be easily assimilated by other phytoplankters and/or bacteria (Glibert and

Bronk, 1994). Furthermore, both nitrogen fixation (Fig. 6) and *Trichodesmium* abundances typically reach high values in this region (Tyrrell et al., 2003; Moore et al., 2009; Fernández et al., 2010). Thus, the supply of light ammonium linked to diazotrophs is probably determining an important fraction of the nitrogen isotopic budget in the equatorial region. The two-end-member mixing model (Montoya et al., 2002) yields an average contribution of this diazotroph nitrogen to $\delta^{15}N_{sp}$ of 62±27% in autumn 2007 and 39±8% in spring 2008 (Table 4). On the other hand, Mouriño-Carballido et al. (2011) estimated that the daily contribution of N_2 fixation to total (N_2 fixation + vertical diffusion of nitrate) input of new nitrogen was 22% in the 2008 cruise. Again, these fluxes represent different time scales, but both suggest that nitrogen fixation account for a relevant fraction of the supply of nitrogen to the euphotic layer in this region, and are consistent with previous experimental measurements.

The diazotroph nitrogen was inefficiently transferred to upper trophic levels, as it accounted for 25±18% in the 40-200 µm size-fraction and 11±3% in the >200 µm size-fraction in 2007 cruise, and for 3±2% in the 40-200µm size-fraction and 29±12% in the >200 µm size-fraction in 2008 cruise (Table 4). *Trichodesmium*, the dominant diazotroph in this region, is toxic to many species of zooplankton (Hawser et al., 1992) and only a few groups of copepods are known to graze it (O'Neil and Roman, 1994). Besides, these groups seem to excrete a major fraction of the ingested nitrogen (O'Neil et al., 1996, Wannicke et al., 2010). Thus, diazotroph nitrogen is preferentially transferred through dissolved pools when *Trichodesmium* dominates the community (Mulholland, 2007).

Meridional variations in $\delta^{15}N$ in the South gyre region

The distribution of $\delta^{15}N$ of suspended particles ($\delta^{15}N_{sp}$) and zooplankton in the South gyre region depicted contrasting trends in 2007 and 2008 (Fig. 6). Even though the vertical distribution of $\delta^{15}N_{sp}$ varied within a range of 6‰, data were <4‰ in all stations (Fig. 5) suggesting that a ^{15}N-depleted source of nitrogen is significantly contributing to the signals. In autumn 2007, the general meridional pattern largely coincided with that described by Mino et al. (2002) in surface waters, but not with those given by Mahaffey et al. (2004) and Reynolds et al. (2007), who found a general increasing trend to the South with values >2‰. The light patch of $\delta^{15}N_{sp}$ in the range −2 to 0‰, which was found between 20º-30ºS in the 2007 cruise (Fig. 6), seems to be a persistent feature also described by Mino et al. (2002) with values close to −1‰, and Reynolds et al. (2007) with values close to 0‰. By contrast, this is the first time that a decreasing trend in $\delta^{15}N$ such as the one depicted in spring 2008 is described in this region.

The flux of atmospheric deposition of nutrients in the South Atlantic Ocean is extremely weak (Gao et al., 2001; Duce et al., 2008), thus we may discard the effect of light atmospheric nitrogen in the

isotopic budget. The small difference in the $\delta^{15}N$ of both zooplankton size-fractions suggests a high coupling between trophic levels, with low isotopic fractionation in the loss of nitrogen by excretion (Checkley and Miller, 1989). The uptake of dissolved organic nitrogen and their inorganic degradation products, originated by the nitrogen fixers and processed by microbes, may explain such coupling, as isotopic fractionation in microbial food webs is generally low (Rau et al., 1990). Mahaffey et al. (2004) suggested that the relatively important dissolved organic nitrogen pool of the South Atlantic could account for the high $\delta^{15}N$ measured in their study. On the contrary, Knapp et al. (2011) found that a long-lived and poorly reactive DON pool in other regions of the Atlantic and Pacific Oceans which could be a source of light ammonium through deamination. However, we propose that the supply of diazotroph nitrogen is significantly determining the observed $\delta^{15}N_{sp}$. The few experimental measurements performed to date in the South Atlantic show that nitrogen fixation is persistent in this region with rates in the range 2 to 50 µmol N m^{-2} d^{-1} (Moore et al., 2009; Fernández et al., 2010; Grosskopf et al., 2012), which are similar to those reported in the equatorial and North gyre region (Fig. 6). This suggests that nitrogen fixation could be responsible of the persistent light patch of $\delta^{15}N_{sp}$ in the South gyre between 10º-30ºS. The contribution of nitrogen fixation to the $\delta^{15}N$ of suspended particles was 49±12% in 2007 and 58±18% in 2008, according to a two-end-member model based on nitrate and diazotrophy (Montoya et al., 2002). Besides, the daily contribution of nitrogen fixation to the total (N_2 fixation + nitrate diffusive flux) input of nitrogen to the euphotic layer was 44% during strong stratification conditions in April 2008 (Mouriño-Carballido et al., 2011).

This diazotroph nitrogen is transferred to upper trophic levels with relatively high efficiency in 2008 and it represented 36±16% of the 40-200µm size-fraction and 40±17% of the >200µm size-fraction (Table 4). Hence, both experimental measurements and estimations seem to agree in that nitrogen fixation could be supporting an important fraction of primary production in the South gyre, despite the fact that the absolute rates of both processes are low.

Conclusions

A persistent and consistent signature of low $\delta^{15}N$ of suspended particles ($\delta^{15}N_{sp}$) is found in the North gyre region (30º-15ºN) in both zonal and meridional transects, which is usually associated with a relevant input of nitrogen fixed by diazotrophs (Mahaffey et al., 2003; Reynolds et al., 2007; Landrum et al., 2011). However, the experimental measurements of nitrogen fixation show modest rates in comparison with other regions of the Atlantic Ocean and do not seem to support this argument. The atmospheric deposition of light nitrogen, which is increasing in the last years, is likely to complete the required supply that produces this depleted $\delta^{15}N$ signal. However, few studies

have addressed the depositional and diazotrophic fluxes together (Baker et al., 2007; Knapp et al., 2011) and further studies are needed to accurately define the strength, frequency and isotopic composition of the atmospheric depositional flux against the flux of nitrogen fixation in the North Atlantic. The equatorial region (15ºN-10ºS) is subject to relatively intense nitrogen fixation throughout the year (Moore et al., 2009; Fernández et al., 2010; Grosskopf et al., 2012) which may explain 40 to 60% of the observed $\delta^{15}N_{sp}$ signal. However, this nitrogen of diazotrophic origin seems to be inefficiently transferred to upper trophic levels. In the South gyre, the low $\delta^{15}N_{sp}$ and the daily estimated contribution of nitrogen fixation to the supply of new nitrogen (Mouriño-Carballido et al., 2011) suggest that diazotrophs can contribute up to half of the nitrogen in phytoplankton at different time scales (Fig. 4). Even though the measured nitrogen fixation rates are low (Moore et al., 2009; Fernández et al., 2010; Grosskopf et al., 2012), their impact in the nitrogen isotopic budget of this region may be large. Hence, a re-evaluation of the importance of diazotrophy in the South Atlantic Ocean is needed through new studies that should address the annual variability in nitrogen fixation rates as well as the distribution and relative importance of the different groups of diazotrophs.

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631 Table and figure legends

632 Table 1. Pearson product-moment correlation coefficient between $\delta^{15}N$ of suspended particles
633 ($\delta^{15}N_{sp}$) and nutrient concentration: ammonium (NH_4) and nitrate (NO_3), in 2007 and 2008 cruises.
634 Numbers in brackets represent the total number of samples used for the analysis.

635 Table 2. Pearson product-moment correlation coefficient between $\delta^{15}N$ of suspended particles
636 ($\delta^{15}N_{sp}$), $\delta^{15}N$ of 40-200µm plankton size-fraction ($\delta^{15}N_{40}$), and $\delta^{15}N$ of >200µm plankton size-
637 fraction ($\delta^{15}N_{200}$) in the latitudinal transects of 2007 and 2008 cruises. ** $p< 0.01$, n=17.

638 Table 3. Two-way factorial ANOVA (region, cruise) of $\delta^{15}N$ of suspended particles ($\delta^{15}N_{sp}$), $\delta^{15}N$
639 of 40-200µm plankton size-fraction ($\delta^{15}N_{40}$), and $\delta^{15}N$ of >200µm plankton size-fraction ($\delta^{15}N_{200}$)
640 in the 2007 and 2008 cruises. DF, degrees of freedom; SS, sums of squares; MS, mean of squares;
641 F, F statistic; p, probability.

642 Table 4. Mean±Standard deviation of the contribution of diazotroph nitrogen to $\delta^{15}N$ of suspended
643 particles, 40-200µm plankton size-fraction ($\delta^{15}N_{40}$) and >200µm plankton size-fraction ($\delta^{15}N_{200}$)
644 according to the two-end-member mixing model proposed by Montoya et al. (2002). The reference
645 zooplankton used in each fraction corresponded to the average of the stations sampled in the South
646 gyre during 2007, where *Trichodesmium* abundance was < 1 trichome L^{-1}, $\delta^{15}N_{40}$ = 4.6‰, $\delta^{15}N_{200}$ =
647 5.9‰. The values of % of diazotroph N above 100 and below 0 were discarded in the calculation of
648 the regions average. Number of samples is indicated in parentheses.

649 Figure 1. Sampling stations during the TRYNITROP cruises on board the BIO 'Hespérides'. White
650 circles represent the autumn 2007 cruise (17 November - 8 December 2007), and grey triangles the
651 spring 2008 cruise (13 April - 2 May 2008).

652 Figure 2. Zonal and meridional vertical distribution of temperature (ºC), salinity and fluorescence in
653 autumn 2007 and spring 2008 cruises. Dashed lines in the temperature panels define the limits of
654 the three major regions identified by the depth of 16ºC isotherm: North gyre, equatorial region and
655 South gyre.

656 Figure 3. Relationship between the $\delta^{15}N$ of suspended particles ($\delta^{15}N_{sp}$) and the particulate organic
657 nitrogen (PON) to chlorophyll *a* (chl-*a*) ratio during the autumn 2007 (a) and the spring 2008 (b)
658 cruises.

659 Figure 4. Zonal and meridional distributions of particulate organic nitrogen of suspended particles
660 (PON) during the autumn 2007 (a, b) and spring 2008 (c, d) cruises. Dashed lines define the limits
661 of the three major regions identified by the depth of 16ºC isotherm in the meridional transect: North
662 gyre, equatorial region and South gyre. In the legend z1 to z6 represent the sampled depths from
663 deeper depth, z1 (DCM), to shallower depth, z6 (5m).

Figure 5. Vertical distribution of $\delta^{15}N$ of suspended particles ($\delta^{15}N_{sp}$) in autumn 2007 and spring 2008 cruises grouped by region: a, b) zonal transect (15º-38º W), c, d) North gyre region (30º-15ºN), e, f) equatorial region (15ºN-10ºS), and g, h) South gyre region (10º-30ºS).

Figure 6. Zonal and meridional distributions of the weighted mean of $\delta^{15}N$ of suspended particles ($\delta^{15}N_{sp}$), the $\delta^{15}N$ of 40-200µm plankton size-fraction ($\delta^{15}N_{40}$), the $\delta^{15}N$ of >200µm plankton size-fraction ($\delta^{15}N_{200}$), and concurrent measured N_2 fixation (Fernández et al., 2010; Fernández et al., 2013) in autumn 2007 (a, b) and spring 2008 (c, d).Dashed lines define the limits of the three major regions identified by the depth of 16ºC isotherm in the meridional transect: North gyre, equatorial region and South gyre.

674 Table 1.

	2007 cruise				2008 cruise			
	All stations	North gyre[a]	Equatorial region	South gyre	All stations	North gyre[a]	Equatorial region	South gyre
NH_4	0.22* [128]	0.55* [54]	0.43** [38]	n.s.	n.s.	n.s.	0.47** [42]	−0.38* [36]
NO_3	n.s.	0.30* [54]	n.s.	n.s.	0.21* [120]	n.s.	n.s.	0.51** [36]

675 ** $p < 0.01$, * $p < 0.05$, n.s. no significance

676 [a] includes the zonal and meridional legs in the North gyre.

677

678 Table 2.

	$\delta^{15}N_{sp}$		$\delta^{15}N_{40}$	
	2007	2008	2007	2008
$\delta^{15}N_{40}$	0.66 **	0.82 **	–	–
$\delta^{15}N_{200}$	0.66 **	0.74 **	0.90 **	0.78 **

679

680 Table 3.

681

		$\delta^{15}N_{sp}$				$\delta^{15}N_{40}$				$\delta^{15}N_{200}$			
	DF	SS	MS	F	p	SS	MS	F	p	SS	MS	F	p
region	2	23.6	11.8	6.3	0.004	26.6	13.3	11.5	0.000	25.7	12.8	15.4	0.000
cruise	1	16.0	16.0	8.6	0.006	4.8	4.8	4.2	0.048	15.9	15.9	19.2	0.000
region *vs*.cruise	2	6.0	3.0	1.6	0.213	20.9	10.5	9.0	0.000	21.7	10.9	13.1	0.000

682

683 Table 4.

684

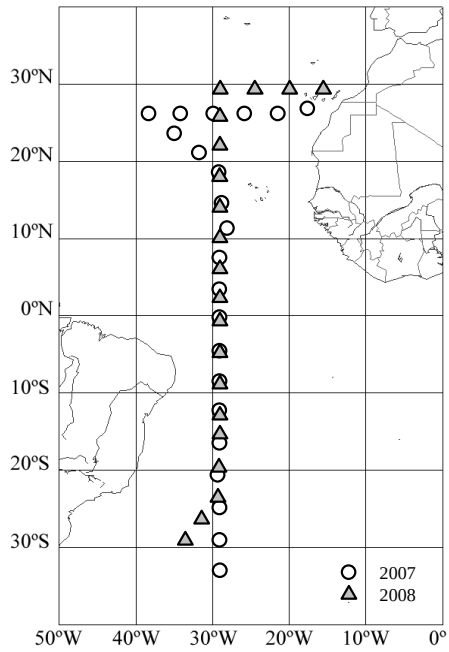
Region	Suspended particles		40-200µm zooplankton		>200µm zooplankton	
	2007	2008	2007	2008	2007	2008
Longitudinal transect	81 (29)	59 (3)	52 (14)	16 (9)	43 (4)	31 (6)
North gyre region	85 (14)	61 (10)	48 (22)	21 (11)	41 (12)	36 (8)
Equatorial region	62 (27)	39 (8)	25 (18)	3 (2)	11 (3)	29 (12)
South gyre region	49 (12)	58 (18)	15 (20)	36 (16)	13 (15)	40 (17)

685

686

687 Figure 1.

688



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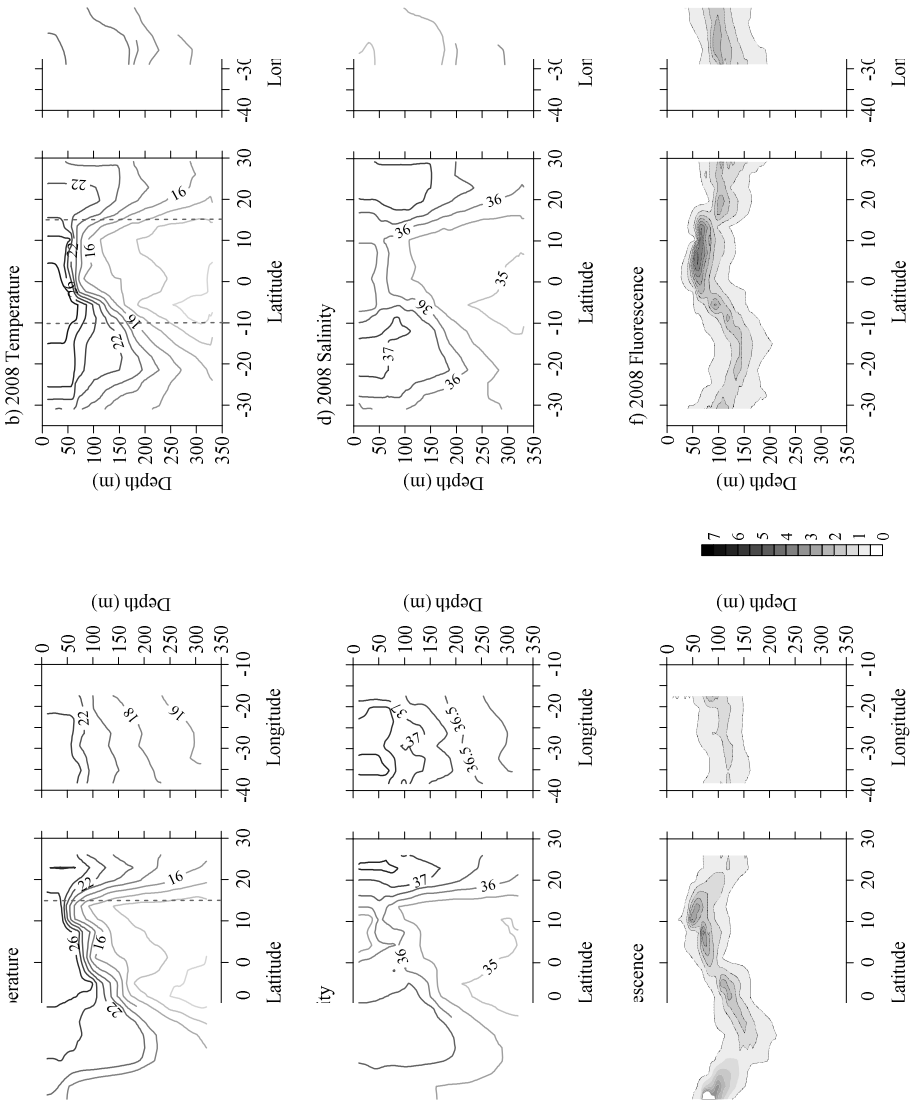
691 Figure 2.



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694 Figure 3.

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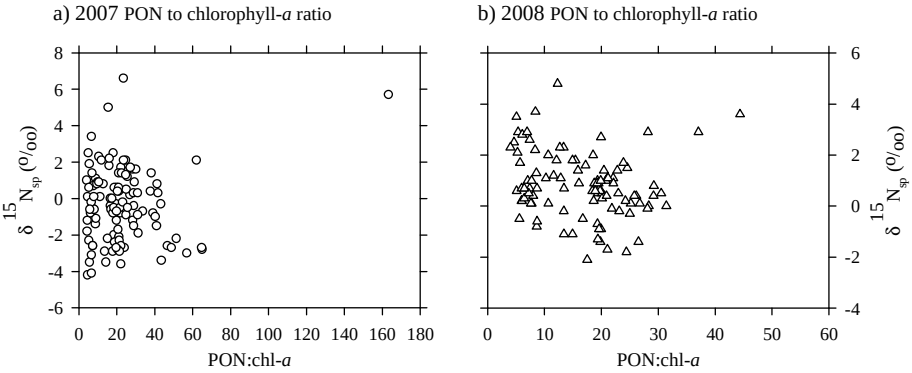

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698 Figure 4.

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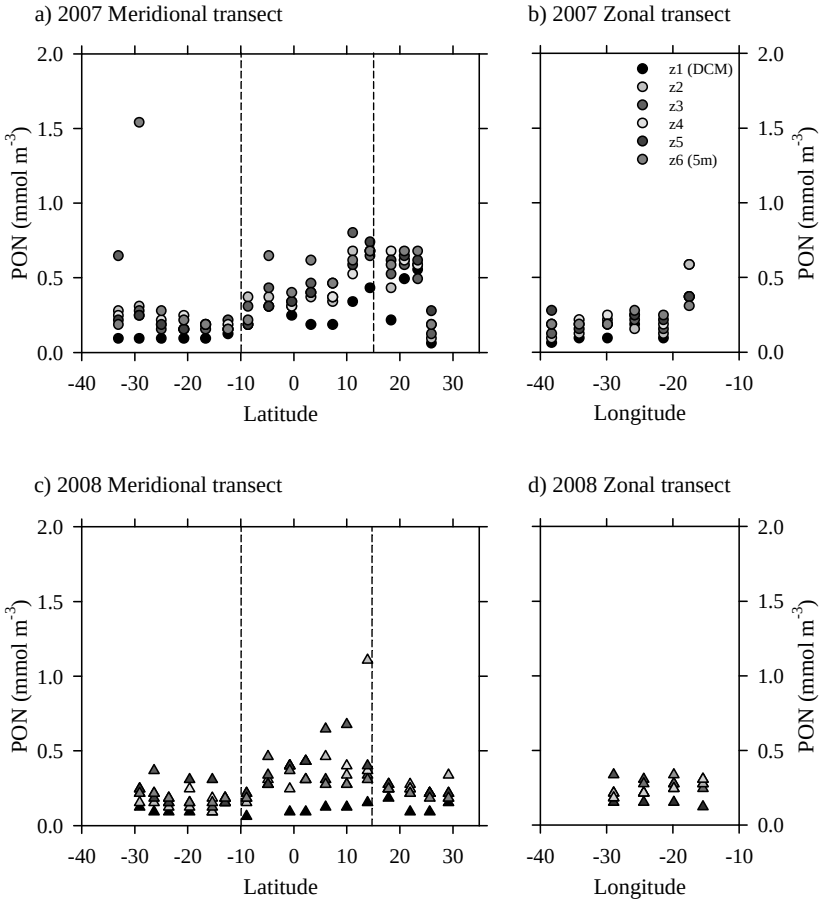

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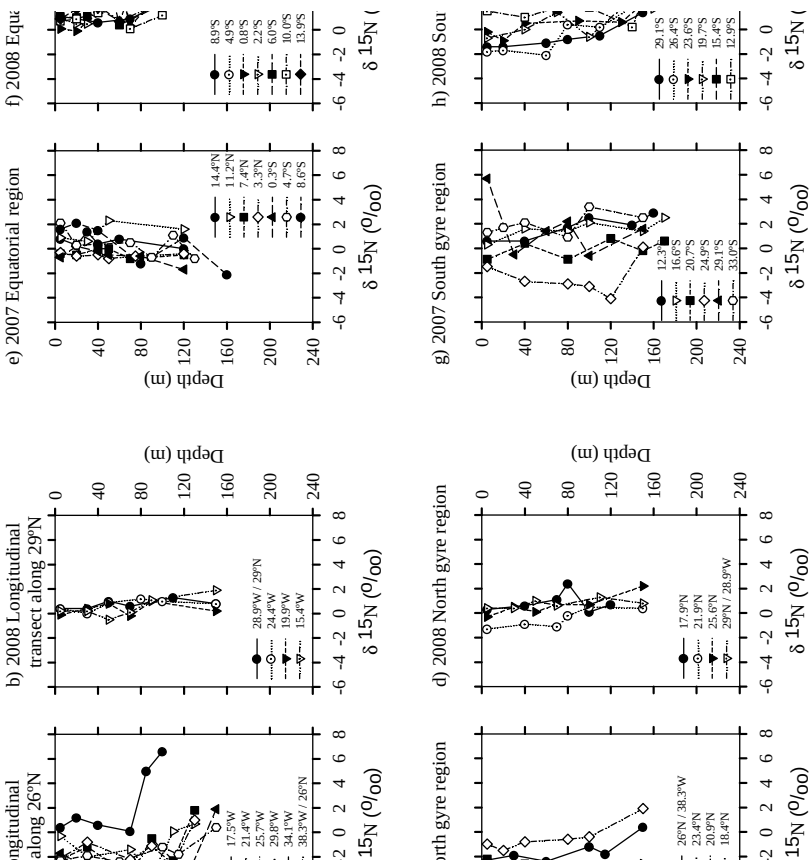
Figure 5.


ngitudinal
along 26°N
17.5°W
21.4°W
25.7°W
29.8°W
34.1°W
38.3°W / 26°N
δ 15N (o/oo)
b) 2008 Longitudinal
transect along 29°N
28.9°W / 29°N
24.4°W
19.9°W
15.4°W
Depth (m)
δ 15N (o/oo)
e) 2007 Equatorial region
14.4°N
11.2°N
7.4°N
3.3°N
0.3°S
4.7°S
8.6°S
Depth (m)
δ 15N (o/oo)
f) 2008 Equa
8.9°S
4.9°S
0.8°S
2.2°S
6.0°S
10.0°S
13.9°S
δ 15N (
rth gyre region
26°N / 38.3°W
23.4°N
20.9°N
18.4°N
δ 15N (o/oo)
d) 2008 North gyre region
17.9°N
21.9°N
25.6°N
29°N / 28.9°W
Depth (m)
δ 15N (o/oo)
g) 2007 South gyre region
12.3°S
16.6°S
20.7°S
24.9°S
29.1°S
33.0°S
Depth (m)
δ 15N (o/oo)
h) 2008 Sou
29.1°S
26.4°S
23.6°S
19.7°S
15.4°S
12.9°S
δ 15N (

706 Figure 6.

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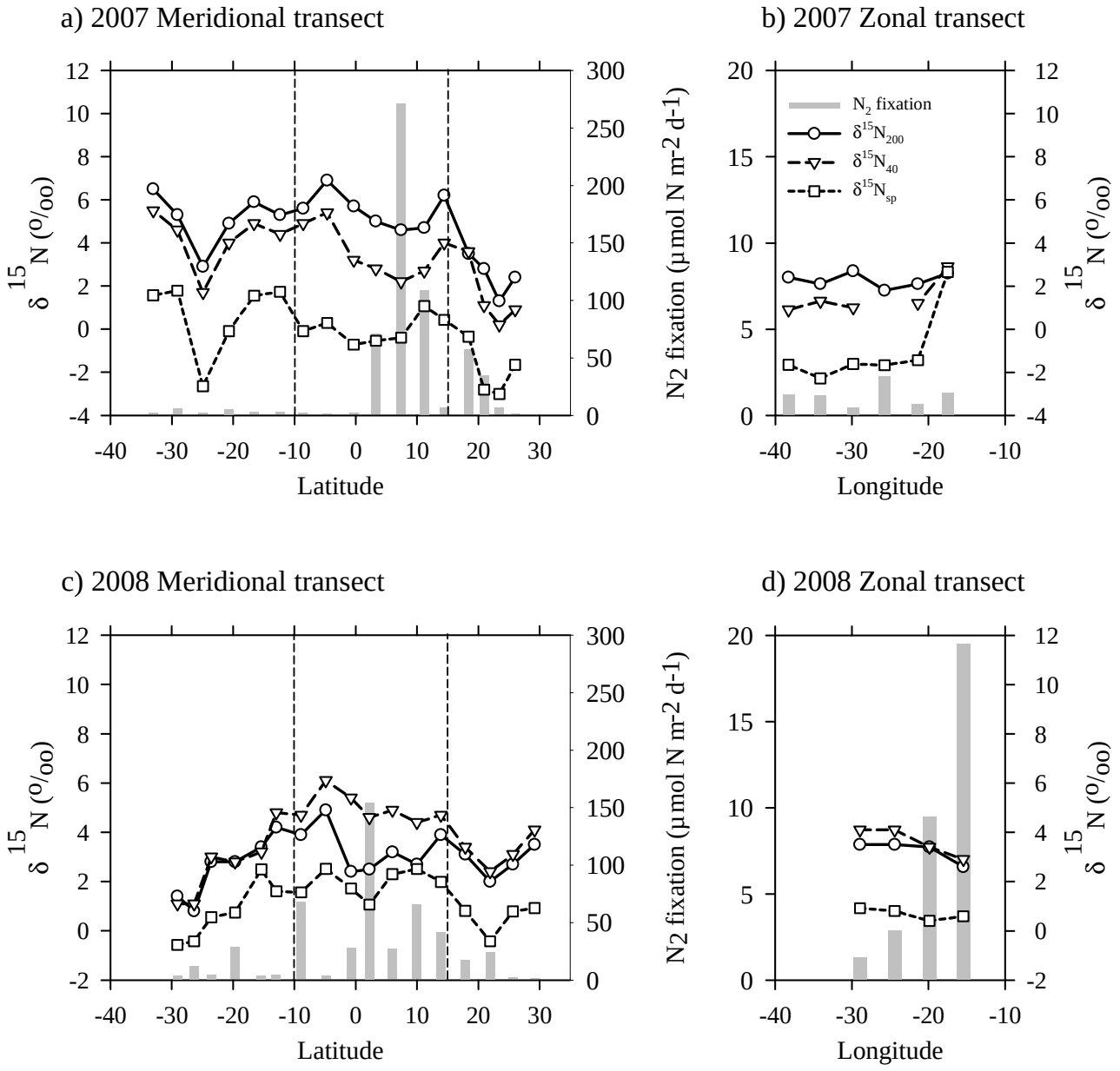

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